HIGGINS

Chemical Study
of Meat Extracts

Chemistry
B. S.

1902

A CHEMICAL STUDY OF MEAT EXTRACTS

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THESIS

FOR THE

DEGREE OF BACHELOR OF SCIENCE

IN CHEMISTRY

COLLEGE OF SCIENCE

UNIVERSITY OF ILLINOIS

1902

UNIVERSITY OF ILLINOIS

June 2nd, 1902

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED A Chemical Study of Meat Extracts.
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A CHEMICAL STUDY OF MEAT EXTRACTS.

HISTORY.

Extract of meat was first described by Proust in 1801, but the method of manufacturing it on a commercial scale is due to Liebig, by whom it was described in 1847. The article has been extensively made and sold under the name of Liebig's Extract since the year 1856, but the Liebig's Extract of Meat Company was not established until 1865. As soon as Liebig had demonstrated that the manufacture of meat extract was a commercial success, numerous concerns were established for the manufacture of preparations similar to Liebig's, while the packing houses already in operation began to include meat extracts among their products. At the present day, extract of meat forms one of the chief products of every packing house, while in South America a number of establishments make meat extracts as their main output.

METHODS OF PREPARATION.

The original method of making extract of meat as described by Liebig, was to treat the finely sliced lean beef, which had been freed from visible fat, with cold water; filter, heat to 50 or 60 degrees C. to coagulate the albumin, refilter, and evaporate to a thick syrup. Liebig admitted, however, that this method was impracticable on a manufacturing scale, and he revised it as follows.

The finely chopped meat is mixed with eight times its

weight of cold water free from gypsum, and the temperature raised to 180 F., or even to boiling. It is then filtered from the residue of meat fibre, coagulated albumen, and fat, and the filtrate evaporated to the desired consistency. Liebig's method has been subjected to a number of modifications, with the result that products are obtained which vary in nature all the way from meat extracts to peptonized foods. In the case of some preparations, gelatin, blood albumen, meat fibre, etc., are added with the view of adding nutriment, or of increasing the concentration.

A process due to Leube (1) is as follows. The finely chopped meat is digested under pressure with water containing hydrochloric acid, thus bringing into solution nearly all the proteid material by conversion into proteoses and peptones. The acid is subsequently neutralized with carbonate of soda, and the solution is then evaporated to extract consistence.

Another method which introduced the use of hydrochloric acid, but in a different manner is that described in a patent, (U. S. 1890, # 10961) by Etienne and Delhaye. The process is as follows: "After removing the tendons and grease, the meat is chopped and mashed, mixed with about half its weight of water, and heated by steam under pressure for an hour at a temperature between 150° and 175° C. A portion of the albuminoid matter is rendered soluble, and goes into solution with the extractives. The residue, when press-

ed forms a friable mass amounting to about 1/3 of the fresh meat used. This residue is treated on the water bath with an equal weight of concentrated hydrochloric acid until the fibro-muscular tissue is quite disintegrated and decomposed, when the liquid is filtered. The insoluble residue is sold as manure. The liquids are neutralized with sodium carbonate, and then contain 'peptone' and sodium chloride in solution. If pure peptone is required, the liquid is decolorized with animal charcoal and dialyzed to remove the salts; but if only meat extract is desired, the liquids from the steam treatment of the meat and the neutralized liquids from the acid treatment, are mixed and evaporated in a vacuum until sufficiently concentrated." (2)

These are but a few of the many processes in use, the details of which are as a rule secrets known only to the manufacturer; but sufficient has been said to give a general idea of the methods used in the manufacture of these preparations, and to throw some light on the composition of certain extracts which are to be found in the market.

CLASSES OF MEAT EXTRACTS.

To find a satisfactory basis for the classification of meat extracts is indeed a difficult task. There are so many varieties of meat extracts upon the market, all having practicall the same qualitative composition, that at best it is only possible to make a very general classification. It is usual, however, to dis-

tinguish four general classes of preparations which have the general nature of "Liebig's extractum carnis"; but it must be remembered that each class represents a large variety of preparations. The four divisions are as follows;

I. Concentrated extracts, having the consistency of a thick syrup, which are usually designated as "Solid Extract of Beef".

II. Liquid extracts, which embrace the so-called "meat juices", "bouillons", "essences of beef," etc.

III. Extracts to which have been added albumen, gelatin, meat fibre, and other such substances not usually found to any extent in true extracts.

IV. "Peptonized extracts", which have been subjected to such processes as those of Leube, and Etienne and Delaye, whereby a large part of the proteids of the meat have been brought into solution.

USES OF MEAT EXTRACTS.

Meat extracts are used extensively in the preparation of soups; for flavoring meats and vegetables; as beef tea; and in numerous other ways. They are frequently recommended by physicians in cases where but little solid food can be consumed. Liebig states that the flavor of meat is due to extractives.
Hence if the insoluble part of any kind of meat be treated with an extract of a different kind, it will acquire the flavor of the latter. In the same manner, vegetable stuffs may be made to taste

like a meat by treating it with the proper extract.

CHEMICAL COMPOSITION.

The composition of meat extract varies between wide limits. Even the same make of extract varies in composition from time to time. Several things are liable to influence the composition of the preparation. The age of the animal, the length of time between slaughtering and the beginning of the extraction, and the use of different kinds of flesh, are sufficient to account for considerable variation in certain of the constituents. Qualitatively, the composition of meat extracts is comparatively uniform, although this is not always the case. The principal constituents of meat extracts may be classed as follows:

I. Water		
II. Mineral Matter		Phosphates of potassium, calcium, sodium
		Chlorides " " and sodium
		Potassium & sodium salts of organic acids
III. Organic Matter.	Proteids	Meat fibre, albumins and globulins syntonin, gelatin and albumoses, peptones
	Meat Bases	Creatin, creatinin, xanthine, hypoxanthine, guanine, carnine, leucin, carnosin.
		Free and combined organic acids, e.g lactic acid and lactates; glycogen and inosite, and unknown constituents

The amounts of these substances vary widely in different preparations. The average composition of solid extract is as follows: Water, 15% to 25 %; ash 20 % to 30 %; organic matter, 50 % to 60 %. The latter comprises 3 % to 6 % gelatin; 1 % to 6 % of albumoses; 0.5 % to 2 % each of soluble albumens, meat fibre, peptones and glycogen; 15% to 20 % meat bases; and 10 % to 26 % of undetermined constituents, including lactic acid. Liquid extracts and peptonized preparations vary from the above percentages chiefly in the content of water, meat meal, albumin and peptones. Thus a certain preparation containing 70 % water, has 16 % soluble albumin; while another article of 17.5 % water, cotains 15 % of meat fibre and coagulated albumin.

FOOD VALUE OF MEAT EXTRACTS.

The food value of meat extract has been a subject of dispute ever since the time of Liebig. At the present time, it is generally accepted that meat extracts are of very little importance as foods, but are valuable as stimulants. The manufacturers of meat extracts frequently claim that their preparations are concentrated forms of food, and contain as much nutritive matter as 30, 40 and 50 times their weight of fresh beef. That such statements are utterly absurd is obvious when it is remembered that fresh lean beef contains about 20 % of nutritive matter, and 75 % of water. Hence by the dessication of four pounds, there would be

obtained one pound of dry meat, of which 30 % is proteid matter, and 20 % is fat, meat bases, and other extractives. Thus the ratio of proteids to extractives in meat is about 4 to 1; in meat extract it is at most 1 to 4. From this it follows at once that the amount of meat extract which would have to be taken to furnish sufficient nutriment to support life is enormously beyond the quantity which could be consumed without upsetting the system, to say nothing of the cost of such an amount.

The fact that meat extract is of so little food value, however, does not detract from its importance as a stimulant. Druitt (3) states that meat extract exerts a rapid and remarkable stimulating action on the brain, and proposes its use as a partial substitute for brandy in cases of great exhaustion attended with cerebral depression. Liebig (4) writes: " Neither tea nor extract of meat is nutriment in the ordinary sense; they possess far higher importance by certain medicinal properties of a peculiar kind. -------They serve the healthy man for the preservation of his health. Taken in proper proportion, they strengthen the internal resistance of the body to the most variable external injurious influences, which combine to disturb the general vital processes, and adjust these latter.-------It is surely a grave offense against all the laws of physiology to compare tea, coffee, and extract of meat with the more common articles of food."

H. Rubner (5) found that the urine of dogs fed on Liebig's

Extract acquired the peculiar odor of meat extract. He concluded that the latter does not contribute to the bodily heat, that the waste of tissue is neither hastened nor retarded by it, and that it passes away unaltered in composition.

Understanding then, that meat extracts are to be classed as stimulants, the value of an extract does not depend on the albuminoid matter, and considerable variation in the quantity of the latter would not affect its properties except as it varied the proportion of meat bases. In passing judgement on a meat extract, therefore, the constituents to be considered are those which possess the stimulative properties; and these are the meat bases, organic acids, mineral salts, and possibly some of the unknown constituents.

METHODS OF ANALYSIS.

Ever since the introduction of meat extracts, their analysis has been a problem which has received the attention of many eminent chemists. Until comparatively recent years, analysis of meat extracts consisted of empirical determinations whereby a comparison between two extracts might be made. In the light of present analyses, however, it is seen that the conclusions and interpretations which were drawn from the older analyses were often erroneous. It is plain, therefore, that correct analyses of meat extracts are necessary to their just valuation. The following

table (6) gives some analyses of extracts sold in New York State in 1882, and serves to show what a crude and unsatisfactory affair it was, upon which judgements of meat extracts were based at that time.

TABLE NO. 1.

Name of Extract	Water	Organic Matter	Ash	Soluble Albumin.	Alcoholic Extract
	%	%	%	%	%
1. Liebig's Extract	18.27	58.48	23.25	0.05	44.11
2. Berger's "	40.65	39.85	19.50	1.11	13.18
3. Starr's "	37.00	55.65	7.35	1.10	10.13
4. Johnston's Fluid Beef	41.20	50.40	8.40	1.17	15.93
5. Grant's Beef Peptone	37.15	54.92	7.93	0.00	20.16
6. Valentine's Meat Juice	54.40	31.85	13.75	0.44	26.32

The only determination of any value in these analyses are the water and ash, and obviously, these are of no value in discovering the nature of the organic matter. The strength of the alcohol used to obtain the results in the last column is not stated, and the determination is without value, in any case, since it is not known what the value of the constituents extracted by alcohol really is.

The difficulties encountered in analyzing meat extracts have to do with the organic matter-- the separation of the nitrogenous constituents from each other and from the non-nitrogenous sub-

stances. The present methods for the quantitative separation of the several proteid substances are far from satisfactory, and in the presence of numerous nitrogenous bases, the separations are still more difficult. It is little wonder, then, when proteids and bases exist in the presence of a large amount of substances whose chemical nature is unknown, that their separation should be a difficult one, and subject to numerous errors.

We shall consider first the separation and estimation of the proteid substances, then the meat bases, and lastly the non-nitrogenous extractives.

The chief proteid substances met with in meat extracts are meat fibre, albumins and globulins, syntonin, albumoses, gelatin, and peptones. (1) Meat fibre is readily determined by dissolving the extract in cold water, filtering and washing, and determining the nitrogen in the residue insoluble in cold water by the ordinary Kjeldahl method. (2) The soluble albumins and globulins are coagulated by heat in acid solution, and may be determined by acidifying the solution with acetic acid, boiling, filtering, and determining the nitrogen in the precipitate. (3) For the determination of syntonin the filtrate from the coagulated albumins is made exactly neutral to litmus, the precipitated syntonin filtered off and the nitrogen in it determined. (4) Albumoses and gelatin together may be determined by saturating the solution with ammonium or zinc sulphate, whereupon the albumoses and gelatin are precipitated; they are

then filtered off, washed with a saturated solution of the precipitant, and the nitrogen determined by the Kjeldahl method. Where ammonium sulphate is used, the ammonia in the sulphate must first be expelled by boiling with magnesium oxide or barium carbonate. On this account, zinc sulphate is to be preferred. (5) Peptones are not precipitated by a saturated solution of zinc sulphate, hence the filtrate from the zinc sulphate precipitation may be used for their determination. The peptones may be precipitated from this solution by saturation with bromine.

These methods are the best in use, but they require careful manipulation in order to get concordant results. Until a few years ago, a process based on fractional precipitation with alcohol was extensively used by a number of chemists, but it has been condemned as too inaccurate. It was claimed that gelatin was precipitated by 50 % to 60 % alcohol, proteoses by 80 %, and peptones by alcohol above 95 %. König and Bömer, however, compared precipitations with 80 % alcohol and with zinc sulphate, and found that the former gave results from 30 % to 50 % too low. They concluded from this and other experiments, that alcohol precipitation is of no value in determining the form in which the nitrogen is present.

The quantitative separation of the meat bases in extracts of meat is even more difficult than the separation of the proteids. The chief bases met with are creatin, and its anhydride, creatinin, xanthine, hypoxanthine, guanine, carnine, leucin and carnosin. In

the ordinary analyses of meat extracts, the bases are estimated together by difference, that is, the nitrogen left after subtracting that in the forms of the several proteids from the total nitrogen, is ascribed to meat bases. This amount is then multiplied by the creatin factor, 3.12, which, if only creatin were present, would be correct. But for creatinin and the bases of the xanthine group, this factor is too high, and therefore gives results above the truth.

In the methods for the direct estimation of creatin and creatinin in meat extract, there is room for considerable improvement. The only method for creatin which can be considered at all accurate is conversion into creatinin by boiling with dilute acids, and precipitation of the creatinin with alcoholic zinc chloride solution. Apparently, however, the other organic substances influence the solubility of the zinc-chloride-creatinin compound, since the writer has been unable to obtain results by this method. Phospho-tungstic acid has also been used for the precipitation of creatinin, but the precipitation is incomplete, and peptones and probably other nitrogen compounds are also precipitated in part.

Xanthine, hypoxanthine, and guanine may be estimated with a fair degree of accuracy by precipitating with ammonio-nitrate of silver, after removal of the proteids by basic lead acetate.

Xanthine, silver may be separated from hypoxanthine and guanine by dissolving in boiling nitric acid of specific gravity 1.10, from which the xanthine silver separates on cooling. Carnine, sarcine, leucine and carnosine are present in beef extract in very small amounts, so that their determination is of little importance, and is seldom made.

The known non-nitrogenous organic constituents of beef extract are lactic acid and lactates, inosite, and glycogen. The methods for the estimation of these substances have been very little studied, and are tedious and wanting in accuracy. Glycogen and inosite occur in very small amounts, and are sometimes entirely absent, while the amount of lactic acid has never been accurately determined.

Having given this brief outline of the methods which have been used in analyzing meat extracts, we will describe in more detail the methods of analysis used in connection with this study.

WATER: The water in solid extracts was determined by drying 2 to 3 grams in lead or platinum dishes in the water oven at 100°C. until constant weight was obtained.

FAT: The fat was determined on the weighed residues from the water determinations made in lead dishes. The latter were rolled up and placed in the Soxhlet extracting apparatus, and extracted with pure anhydrous ether for 24 hours. The ether extract was evaporated, dried at 104° and weighed.

ASH: " Official Method": A weighed sample of 2 to 3 grams is charred in a platinum dish at a temperature below redness, until completely carbonized. It is then extracted several times with boiling water, decanting each extraction through an ashless filter; The filter and charcoal is returned to the dish, and ignited at full redness until white. The water extract is then transferred to the dish, evaporated to dryness on the water bath, and finally heated over the free flame below redness, cooled and weighed. The volatilization of the sodium and potassium chlorides is thus prevented.

TOTAL NITROGEN: About 1 gram of solid extract was introduced into a Kjeldahl flask, and the nitrogen determined by the ordinary Kjeldahl process.

For the remaining determinations about 10 grams of solid extract was dissolved in water, and diluted to exactly 500 cc.

MEAT FIBRE: This solution is filtered, and the nitrogen determined in 25 cc. portions of the filtrate. The difference between the nitrogen thus found and the total nitrogen, is ascribed to the meat fibre.

TOTAL SOLIDS: 25 cc. of the solution is evaporated on the water bath, and heated to 100 C. in the water oven, until constant in weight.

ASH: The ash is determined by the Official Method on the weighed total solids.

ALBUMINS and GLOBULINS: 350 cc. of the solution is acidified with acetic acid (12 cc. N/10 CH_3COOH was found sufficient) and boiled for half an hour or more. The coagulated albumins and globulins are filtered off, washed with hot water, and the nitrogen determined in the precipitate by the Kjeldahl Method.

SYNTONIN: The filtrate from the above is made exactly neutral to litmus by addition of dilute caustic soda solution, allowed to stand two hours, filtered, and the nitrogen in the precipitate determined.

PROTEOSES and GELATIN: The filtrate from the syntonin is divided into two equal parts, and each evaporated to 50 cc. or less. On cooling, 3 or 4 cc. of dilute sulphuric acid is added, and the solutions then saturated with pulverized zinc sulphate. After standing about 6 hours, the precipitate is filtered off, washed with a saturated solution of zinc sulphate, and the nitrogen in it determined.

PEPTONES: The filtrate from the zinc sulphate precipitate is diluted with an equal volume of water in a Kjeldahl flask, and liquid bromine added with constant shaking until it ceases to be absorbed, and a globule of about a cubic centimeter remains at the bottom of the flask. After standing for twelve hours or more, the solution is filtered, washed with saturated bromine water, the filter and contents returned to the flask, and the nitrogen determined.

For all the proteids, the nitrogen found is multiplied by

the factor 6.25 to obtain the amount of proteid.

FLESH BASES: The sum of the nitrogen in the forms of the several proteids is subtracted from the total nitrogen in the preparation, and the difference multiplied by the creatin factor, 3.12, to obtain the amount of bases.

COMPILATION AND COMPARISON OF ANALYSES.

TABLE No. 2,(7)

Name of Extract.	H_2O	Organic Substance	Salts	The organic matter contains				
				N as albumen	albumen	N as peptone	peptone	N as meat bases
	%	%	%	%	%	%	%	%
1.Kemmerich's Ext.	20.95	60.81	18.24	1.258	7.86	2.308	14.42	6.167
2.Liebig's "	19.33	57.52	23.25	0.848	5.30	0.284	1.77	7.782
3.Murdock's Liquid Food	83.61	15.83	0.56	2.066	12.91	0.037	0.23	0.187
4.Valentine's Meat Juice	59.07	29.41	11.52	0.292	1.82	0.760	4.75	1.448
5.Johnston's Fluid Beef	49.49	45.32	5.19	2.824	17.65	2.287	17.73	1.394
6.Benger's Peptonized Beef Jelly	89.68	9.43	0.89	0.386	2.41	0.741	4.63	0.422
7.Savory & Moore's Fluid Beef	27.01	60.89	12.10	0.869	5.43	0.543	2.66	7.472
8.Brand & Co's. Essence of Beef	89.19	9.50	1.31	0.360	2.25	2.250	6.05	0.154
9.Carnick's Peptonoids	6.75	87.57	5.50	9.060	56.62	56.62	6.93	0.100

Arranging these preparations according to their percentage of nitrogen in the form of meat bases, which is the index of their stimulating power, and consequently of their value, we find that 100 parts of Liebig's Extract contains as much nitrogen in the form of meat bases as

126	parts of	Kemmerich's Extract
537	" "	Valentine's Meat Juice
558	" "	Johnston's Fluid Beef
1844	" "	Benger's Peptonized Beef Jelly
4161	" "	Murdock's Liquid Beef
5083	" "	Brand & Co's. Essence of Beef
7782	" "	Carnick's Peptonoids.

The analyses tabulated on the following page, in Table No. 3 were made in connection with this investigation by the methods already described. Several analyses similar to these have been made of Armour's and Liebig's Extracts, but we know of no previous analyses of this nature, of Swift's, Nelson Morris & Co's., or Cudahy's Extracts, which are prominent American makes.

Marked similarity is noticed between Armour's and Liebig's Extracts, although the one is of American manufacture, and the other South American. There is also a strong similarity existing among the other three extracts, especially in the per cent of ash. It seems probable that two different processes are used in the manufacture of Armour's and Liebig's on the one hand, and of Swift's, Cuda-

hy's and Nelson Morris' on the other.

TABLE No. 3.

Analyses of Representative Meat Extracts.

Name of Extract.	Class	Moisture	Total Solids	Ash	Fat	Total Nitrogen	Water Solution: filtered.											
							Total Nitrogen	Meat fibre	Albumins & Globulins	Proteoses	Syntonin	Peptones	Flesh Bases	Total Solids	Ash	Unknown by diff.
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Liebig's	Solid	18.6	81.4	21.70	0.30	9.19	8.95	1.50	?	11.75	0.00	0.65	19.97	80.75	21.65	25.2
Armour's	Solid	18.3	81.7	22.48	0.25	8.55	8.25	1.87	0.70	13.31	0.00	1.55	18.31	81.5	23.4	23.1
Armour's (5# jar)	Solid	17.3	82.7	23.28	0.50	8.47	8.36	0.69	0.93	8.87	0.00	1.25	18.81	79.49	22.78	26.0
Nelson Morris'	Solid	—	—	30.95	—	7.75	7.46	1.81	0.87	15.56	0.00	2.15	14.00	82.45	30.72	17.33
Swift's	Solid	—	—	—	—	6.55	6.35	1.25	0.62	5.44	0.00	1.03	16.30	83.32	31.50	27.18
Cudahy's	Solid	—	—	—	—	7.40	7.40	0.00	1.00	9.69	0.00	1.35	17.12	70.56	29.57	11.84

UNDETERMINED CONSTITUENTS.

In Table No. 3, we find a column labelled " Undetermined , by difference". This undetermined material varies from 11 % to 25% in the above analyses, which are typical analyses of solid extracts. In 1899 Mr. Thomas McFarland, of the Inland Revenue Department of Canada, published analyses of commercial extracts which showed 22 % to 26 % of undetermined material. He made no attempt, however, to discover the nature of these unknown substances. The first work done with a view of separating and identifying this unknown material was by Emmett (9) in this laboratory in 1900-1901. Emmett worked with meat broth prepared by himself from fresh beef, and found that it contained about the same amount of unknown substances as commercial extracts. He sought to identify these unknown compounds by separating from them the known constituents, but was unable to make a complete separation. The process that he used was, briefly as follows.

He extracted the broth in a syrupy condition with 95 % alcohol, thus removing a considerable part of the proteids, together with some creatin and mineral matter, but also a part of the unknown. The 95 % alcohol extract contained about 45 % of undetermined substances, 36 % flesh bases, 5 % proteids, and 13 % ash. To remove the ash and the nitrogen compounds, he first neutralized with baryta water, filtered off the precipitated phosphates, and added a saturated solution of mercuric chloride to the filtrate. After standing

for 24 hours, the precipitate was filtered off, and the filtrate, after it had been freed from mercury by means of sulphuretted hydrogen, was analyzed. The analysis showed 14 % less of flesh bases, 2 % less ash, and an increase of 10 % in unknown constituents. He next attempted to remove more creatin and hydrochloric acid by heating with acid for several hours, and then adding mercuric oxide . This treatment removed 6 % creatin, 2 % ash and increased the amount of undetermined constituents to 75 %. The solution was next treated with silver oxide to remove hydrochloric acid and more creatin, but this step proved unsatisfactory, since considerable silver went into solution. Various mixtures of absolute alcohol and ether were next used, and finally a mixture consisting of four parts alcohol (absolute) and five parts of anhydrous ether was found to remove the greatest amount of nitrogen and mineral matter. The filtrates were evaporated to a syrup, but all attempts to crystallize them proved useless. Emmett made complete analyses of these products, however, and calculated a formula for one---- $C_{11}H_{21}NO_6$. He made numerous tests with various reagents, and the results seemed to indicate that the material possessed alkaloidal properties. He concluded that, instead of the unknown material being non-nitrogenous, it consisted of a substance or substances ~~of~~ of alkaloidal nature with a small nitrogen content.

At the same time that Emmett was making the study of meat

broth, Lyman (10) was trying to solve the same problem with regard to commercial beef extracts. He did not proceed as far as Emmett, however, and since his methods of procedure were somewhat similar to those Emmett employed, we will pass by his work with this brief reference.

In the summer of 1901, Dr. H. S. Grindley repeated in part the work of Emmett, with a number of modifications. Armour and Company's Solid Extract of Beef was used throughout. After the precipitation with mercuric chloride, the process varied considerably from Emmett's procedure, but the method was not successful in separating the unknown constituents in a state of purity. The work, however, was not carried to completion, and on that account no definite conclusions could be drawn.

RESULTS OF THIS STUDY IN DETAIL.

From the preceding statements in regard to the extent of our present knowledge concerning meat extracts, it is seen that the separation and identification of the unknown constituents is a problem whose solution would be of the utmost interest and value. It has also been pointed out that the previous attempts which have been made in this direction, although paving the way for later investigation, have, nevertheless, fallen short of the desired end. For this reason, the principal aim of the present study has not been exclusively the identifying of the unknown constituents, although indirectly considerable light has been thrown upon that question. It was thought that a more detailed investigation than had been previously made of some of the unknown costituents, especially of the non-proteid nitrogenous substances, would be of value in two ways. (1) It would establish the percentages of the various organic bases in the extract, and (2) it might be instrumental in discovering a part, at least, of the unknown material.

The first work to be done, however, was to make complete analyses of four American extracts and one South American extract by the methods already described. The results of these analyses have already been given, and need not be repeated here.

When these analyses had been completed, the detailed study of the principal non-proteid organic substances was undertaken.

Inasmuch as the process is of considerable length and cumbersome to describe, a general description will first be given, and then a diagram of the process in detail will follow.

A sample of about 28 grams was removed from a five pound jar of Armour's Solid Extract of Beef (Shield Brand), and dissolvin about 300 cc. of nitrogen free water; it was then boiled and filtered to remove the meat fibre and coagulated albumin. The filtrate was precipitated with a solution of basic lead acetate (a). After 48 hours, the precipitate was filtered off and washed with water containing 5 % of the basic lead acetate solution. The precipitate should contain almost all the albuminoid substances, phosphoric and uric acids, carnine, inosite, and glycogen.

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(a) The basic lead acetate solution was prepared as follows: To a solution of 340 grams of pure lead acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$, in hot water, was added, 200 grams of lead oxide, PbO, and the mixture boiled for ahalf hour. It was then diluted to two litres, shaken thoroughly, and filtered.

The filtrate comprises some of the peptones, which are but partially precipitated by basic lead acetate; much of the mineral matter and, excepting carnine, the organic bases and acids. The filtrate was freed from lead by means of hydrogen sulphide, diluted to definite volume, and analyzed for total solids, ash, and the total nitrogen. The remainder of the solution was evaporated to about 25 cc., and allowed to crystallize for several days. The crystals were filtered off, washed, dried and weighed. Determination of their nitrogen content showed them to be pure creatin. The weight of the creatin thus obtained amounted to only 7 % of the creatin calculated from analysis of the solution by multiplying the nitrgen by 3.12. The filtrate from the creatin crystals was diluted to definite volume, and divided into two equal parts, Part I and Part II.

Part I was made faintly alkaline with ammonia, and precipitated with a solution of ammonio-nitrate of silver (a). After two hours, the precipitate was filtered off, and washed with dilute ammonia water.

(a) The ammonoi-nitrate of silver was prepared by dissolving 26 gr. pure silver nitrate in 25 cc. of water, adding ammonia until the precipitated Ag_2O just redissolved, then diluting to 100 cc.

The precipitate should consist of the silver compounds of xanthine, hypoxanthine and guanine, besides silver chloride. It was dissolved in boiling nitric acid of specific gravity 1.10, the insoluble silver chloride being filtered off and washed with some of the boiling solvent. The filtrate was allowed to stand six hours, whereupon light flaky crystals consisting of the silver compounds of hypoxanthine and guanine, separated. By adding ammonia in slight excess to the mother liquor, the silver compound of xanthine was precipitated as a brown flocculent precipitate. Both these products were decomposed with hydrogen sulphide, the silver sulphide filtered off, and the filtrate, after boiling to remove hydrogen sulphide, diluted to definite volume. The solutions should now contain only the hypoxanthine and guanine, in the one, and the xanthine in the other. The total solids in aliquot portions of each were accordingly determined, thus determining the per cent of hypoxanthine and guanine, and of xanthine. The first two were not separated from each other, but were estimated together.

Part II was evaporated to a thin syrup, and allowed to crystallize further for about a week. The crystals were weighed and amounted to a little over one-half of the amount of creatin crystals first obtained. It is quite probable, as will be seen later, that they also are pure creatin. The mother liquor from these last crystals was diluted to 50 cc. and analyzed for total solids, ash and nitrogen.

Having thus disposed of the filtrate from the basic lead acetate precipitate, the latter itself was suspended in hot water while still moist, and decomposed by passing hydrogen suplhide for about 36 hours in all. The lead sulphide was filtered off with some difficulty, and the filtrate, after boiling to expel the hydrogen sulphide, was precipitated with a solution of neutral lead acetate. After standing a few hours, this was filtered off and washed by suction. The filtrate from this precipitate, therefore, contains those substances which are precipitated by basic lead acetate, but not by the neutral salt. Theses are glycogen, inosite, carnine, and some of the proteids. The substances precipitated by the neutral lead acetate, are, in addition to the proteids, the phosphoric and uric acids. The neutral lead acetate precipitate was accordingly decomposed with hydrogen sulphide, filtered and washed with hot water, and the filtrate, after boiling to remove the excess of hydrogen sulphide, diluted to 250 cc. Analysis of this solution for total solids, ash and total nitrogen showed 73 % of the total solids still undetermined. After analysis, the remainder was acidified with hydrochloric acid, evaporated to about 20 cc., and allowed to stand for six days. The deposit of uric acid, which should separate by this treatment, was too small to be weighed.

The filtrate from the neutral lead acetate precipitate was now freed from lead by passing hydrogen sulphide, the lead sulphide removed by filtration, and the filtrate evaporated to about 15cc.

Two volumes of 94 % alcohol were added, and the solution allowed to stand for one week. This should precipitate glycogen, inosite, and carnine, if present, and possibly some gelatinoid matter. The precipitate was filtered off and washed twice with 60 % alcohol. It was then extracted with hot water, which dissolved only a part of it. The residue was extracted with hot 1 % hydrochloric acid solution, which dissolved nearly all of it. Both the water and hydrochloric acid extracts were evaporated in platinum on the water bath and tested for inosite and glycogen. The results were negative. The precipitate caused by the 60 % alcohol, therefore, must have been some gelatinoid substance, not precipitated by the neutral lead acetate. The precipitate was too small for further study, however, and the work stopped here. On the next page is a detailed diagram of the above processes, followed by a tabulation of the results and conclusions which were reached by this method.

DETAILED DIAGRAM OF "BASIC LEAD ACETATE PROCESS."

Weight of Sample 28.325 grams.

The sample, dissolved in 300cc. water, was boiled, filtered and washed.

A, filtrate: Basic lead acetate added; allowed to stand 48 hrs. Filtered.

B, filtrate; remove Pb with H_2S, filter

C, ppt. Decompose w. H_2S; filter.

B_2, filtrate; diluted to 500cc. Analyzed for total solids, ash and Nitrogen. Remainder, 390cc., evaporated to 25 cc. crystallized for 6 days. Filtered.

C_2, filtrate; expelled H_2S by boiling. Precipitated with neutral lead acetate. After several hours, filtered.

B_{2c}, crystals of creatin, .227 g.

B_{2a}, filtrate, divided into 2 equal pts. B_{2aI} and B_{2aII}.

C_3, ppt. decompose w. H_2S. Filter.

C_4, filtrate; remove Pb w. H_2S, boil, dilute to 100cc. Analyze for total solids, N, ash, evaporate remainder to 15cc. add 30cc. 94% alcohol. After 1 week, filter.

B_{2aI}. Ppt w. ammonio-silver nitrate; after 2hrs filter.

B_{2aII}, evaporate to 10 cc. Allow to stand for 6 days, & filter.

C_5, filtrate, boil, dilute to 250cc. Determine total solids, ash and N. Evaporate remainder, 175cc., to 25 cc., acidify w. HCl, allow to stand 6 days & filter.

X, ppt. Dissolve in boiling HNO_3, sp. g. 1.1 and filter

II_1 crystals. Dry weigh & save.

II_2 filtrate dilute to 50cc., & determine total solids, ash & N.

Z, filtrate; allow to stand 6 hours, filter.

C_6, ppt. Deposit of uric acid too small to weigh.

C_{4a}, ppt. Ex't w. hot water, & then w. 1% HCl. Tested each for inosite & glycogen. Results negative.

W, ppt. decomposed w. H_2S filter.

T, filtrate, add a slight excess of NH_3, and filter.

S, ppt. Decompose w. H_2S, & filter.

V, filtrate. Boil, cool, dilute to 50cc & determine total solids.

R, filtrate; boil, cool dilute to 50 cc. Determine total solids and ash.

RESULTS AND CONCLUSIONS DERIVED FROM THE BASIC LEAD ACETATE PROCESS.

ANALYSES.

Label	Total Solids (grms)	Per cent of Total Solids				
		Ash	N	N x 3.12 Bases	N x 6.25 Protein	Unknown by diff.
B_2	18.20	32.79	----	29.16	---	38.05
V	0.428	0.00	----	----	---	----
R	0.055	0.00	----	----	---	----
II_2	6.727	34.04	8.50	26.52	---	39.44
C_5	3.207	8.00	2.94	----	18.40	73.60
C_4	2.807	16.38	6.90	----	43.15	40.47

The difference between the percents of bases in B_2 and II_2 is 2.64 %. The actual weights of crystals separated from B_2 and II_2 and from B_2, calculated to per cent on the total solids, is 2.63 %. The recrystallized product which was separated, therefore, must have been entirely creatin. The hypoxanthine and guanine, as represented in V, calculated to the meat extract, give 3.8 %. The xanthine, in R, amounts to 0.5 %, thus making the total amount of xanthine bases 4.3 %.

Glycogen and inosite were found to be absent, and only a trace of uric acid was present.

The results obtained in the above processes seemed to indicate

that a repitition of the process with a much larger sample, and with several modifications, would be of value. Accordingly, a sample of 175 grams was dissolved in about two litres of water, and precipitated with basic lead acetate as before. Inasmuch as the general procedure is the same as in the first case, the modifications and differences only will be mentioned.

The filtrate from the decomposition of the basic lead acetate was first analyzed. It showed the following composition:

	Grams	%
Total Solids	33.44	
Protein (N x 6.25)	16.12	47.9
Ash	9.60	28.7
Undetermined (by difference)	7.72	23.4
		100.0

Instead of precipitating now with neutral lead acetate, as previously, the phosphoric and hydrochloric acids were neutralized with lead carbonate, filtered and washed with cold water. The precipitate was decomposed with hydrogen sulphide, and the resulting solution, after removal of the lead sulphide, was analyzed:

	Grams	%
Total Solids	10.40	
Protein (N x 6.25)	1.40	13.47
Phosphoric acid, H_3PO_4	7.65	73.60
Undetermined (by difference)	1.35	12.93
		100.00

The filtrate from the precipitate produced by lead carbonate was then treated with neutral lead acetate, but only a very slight turbidity was produced. On standing several days, however, it increased to an appreciable amount and was filtered off, decomposed with hydrogen sulphide, filtered, and diluted to 100 cc. for future analysis. The filtrate from the neutral lead acetate was freed from lead by hydrogen sulphide, filtered, boiled, diluted to 500 cc. Analyses of this solution showed the following composition:

	grams	%
Total Solids	12.95	
Ash	1.00	7.70
Protein (N x 6.25)	9.22	71.20
Undetermined(by difference)	2.73	21.10
		100.00

This was one of the last analyses made, and time prevented further study of this solution.

We will return now to the filtrate from the basic lead acetate precipitate. After removal of the excess of lead as sulphide, the solution was concentrated on the water bath to about 100 cc. and allowed to stand for over a week in the refrigerator. A considerable quantity of small crystals separated, which were filtered off by suction, redissolved in water, diluted to 500 cc., and analyzed, with the following results:

	grams	%
Total Solids	17.45	
Ash	4.61	26.42
Alkalinity of ash		
Calculated to K_2CO_3	(1.306)	(7.48)
" " $KC_2H_3O_2$	(1.856)	(10.63)
Loss by ignition	0.550	3.15
Meat bases(N x 3.12)	8.08	46.35
Undetermined (by difference)	4.21	24.08
		100.00

The remainder of the solution was thrice recrystallized, and the crystals then purified were found to contain 31.95 % N

Theoretical for creatin 32.06 % N

It is evident that they were pure creatin.

The filtrate from the first crop of crystals was also diluted to 500 cc. and analyzed, with the following results.

	grams	%
Total Solids	92.70	
Ash, including $KC_2H_3O_2$	37.03	39.98
Peptones, by bromine	3.01	3.25
Creatin and other bases	22.78	24.60
Undetermined by difference		32.17
		100.00

An attempt was made to determine the creatin in 25 cc. of this solution by preipitation with alcoholic zinc chloride

solution. The method for determining creatinin in urine was used. The solution was rendered slightly alkaline with calcium hydrate and a solution of calcium chloride added. These reagents did not produce a precipitate. The solution was then evaporated on the water bath to about 20 cc., and an equal volume of absolute alcohol added, and then 60 cc. of 94 % alcohol. The alcoholic solution was allowed to stand 12 hours, then filtered from a small sticky precipitate which contained 0.1 % of nitrogen. The filtrate was treated with 10 cc. of a 20 % solution of zinc chloride in 94 % alcohol, and allowed to stand 24 hours. Only a small sticky precipitate was produced, which quite obviously was not the zinc chloride creatin compound. Thinking that perhaps no creatinin was present, it was attempted to determine the creatin on another portion of the same solution by boiling with hydrochloric acid for several hours, neutralizing with sodium hydroxide, and proceding as before. The same results were obtained. It was thought from these results that there might be something wrong with the method. Therefore, .1500 gram of pure creatin, containing 31.95 % of nitrogen, was dissolved in water, acidified with 5 cc. dilute hydrochloric acid and boiled for two hours. The solution was then neutralized with caustic soda, and proceeded with as above. The zinc chloride precipitate was filtered off, and the nitrogen in it determined by the ordinary Kjeldahl method. The nitrogen multiplied by 3.12 gave .7518 grams of creatin precipitated, which was 50.1 % of the

of the amount used. Whether any signif cance is to be attached to the fact that exactly one half of the creatin was precipitated, I do not know. However, the point was established that some of the creatin may be precipitated by this method, and it was concluded that the reason for not obtaining a precipitate with alcoholic zinc chloride in the case of the extract solution, was on account of the presence of other organic material which rendered the zinc-chloride-creatin in more soluble, or in some other way prevented precipitation.

The remaining 425 cc. of this solution was evaporated to a thick syrup and placed on ice for several days. No further crystallization occurred, however, and it was diluted to 250 cc. as a stock solution from which aliquot parts could be taken for further investigation. Twenty-five cc. of this solution contained 7.8795 grams of solid matter.

The xanthine bases were determined in this solution by two methods; viz., (1) precipitation as the cuprous compounds, and (2) as the silver compounds. (1) 25 cc. of the solution was heated to boiling on a hot plate, and to the boiling solution was added 20 cc. of a saturated solution of sodium acid sulphite, and 20 cc. of a 13 % solution of copper sulphate; it was then boiled for a few minutes, and 5 cc. of a 10 % solution of barium chloride added in order to promote settlement by mechanical entanglement with the barium sulphate formed. After standing on the water bath for

two hours, it was filtered by suction, washed with water at 50°C., and the nitrogen in the precipitate determined. The per cent of nitrogen as peptone was subtracted, and the result multiplied by 2.6, the mean factor for the xanthine bases.

(2) To another 25 cc. portion, ammonia was added until slightly alkaline, and then a solution of ammonio-silver nitrate. After standing for 15 hours, it was filtered and washed by suction, and the nitrogen in the precipitate determined by the Kjeldahl method. The nitrogen was multiplied by 2.6 to obtain the per cent of xanthines. The results do not agree at all with those of the first method, as is seen below.

(1) Xanthine bases by	$CuSO_4$	0.94 %
(2) " " "	$(NH_3)_xAgNO_3$	3.28 %

The latter method is the better, however, since xanthine itself is not precipitated in the former.

Precipitation by means of phospho-tungstic acid was next tried. To a 10 cc. portion of the stock solution was added 5 cc. of dilute sulphuric acid, and 110 cc. of the phospho-tungstic acid solution (prepared by the Official Method). The precipitate was allowed to settle 3 days, when it was filtered off by suction and washed with the phospho-tungstic acid solution. The filtrate was a deep blue color. The filter and contents were thrown into a Kjeldahl flask, and the nitrogen determined.

Nitrogen in the precipitate	2.34 %
" as peptones	0.52 %
" " creatinin	I.82 %
Creatinin (N x 2.69)	4.86 %

It was thought that the amount of nitrogen liberated by treatment with hypobromite solution would be of value in determining the form in which the nitrogen existed, and therefore, an experiment to this end was performed. The sodium hypobromite solution was prepared just before using by adding two cc. of bromine to to 25 cc of a caustic soda solution. The hypobromite solution was placed in a 50 cc. flask, and 5 cc of the solution under investigation was introduced in a small tube, and the flask connected with the nitrometer. The experiment was performed as in the analysis of urine. The amount of nitrogen evolved was calculated to the total nitrogen in the solution. Two experiments gave the following results:

(1) 24.8 % of the total nitrogen was evolved.

(2) 23.7 % " " " " " " .

Assuming that all of the nitrogen comes from creatin, then, since the alkaline hypobromite treatment liberates two thirds of the nitrogen in creatin, 37 % of the total nitrogen is in the form of creatin. Calculating to per cent of solid matter, the creatin amounts to 9.8 % on the above assumption. Let us now sum up the nitrogenous constituents, and see what remains. The figures are in terms

of per cent of the total solids of the solution.

	% Nitrogen	Corresponds to	%
By NaBro treatment	3.15	creatin	9.80
Precipitated by bromine	0.52	peptones	3.25
" " $WO_3.P_2O_5$	2.34	creatinin	4.86
" " $(NH_3)xAgNO_3$	1.21	xanthines	3.28
Totals	6.70		21.19
Total Nitrogen present			8.40
Nitrogen not accounted for			1.70

which last is about 5 % of the undetermined matter. From these figures, it would seem not improbable that the unknown material contains a small percentage of nitrogen, which is in harmony with Emmett's conclusions.

The assumption, however, that creatin is the only source of the nitrogen liberated by the hypobromite reagent is scarcely allowable, for creatin evolves one half of its nitrogen by this treatment. Making allowance for this in the above table, the per cent of creatin would be diminished by about 2.5 % and the nitrogen unaccounted for would be accordingly increased.

THE ACIDITY OF MEAT EXTRACT.

The acidity of meat extract is a question upon which it was thought worth while to spend some little time. No determinations of the amounts of free acids in meat extract have been found in the literature.

It was first attempted to determine the free acid by titration with standard alkali. A small sample of Armour's Extract was dissolved in water, diluted until practically colorless, and titrated with decinormal sodium hydrate, using phenolpthalein for an indicator. The titration, calculated to lactic acid in the sample amounted to 16.5 %. When litmus was used as an indicator, however, only half this amount was obtained. No conclusions, of course, could be drawn from such variable results.

Another sample of about 32 grams was dissolved in water and diluted to 500 cc. A portion of 100cc. was decolorized with animal charcoal, and then titrated with decinormal alkali, using phenolpthalein. The titration was equivalent to 25 % lactic acid. Fifty cubic centimeters of the 500 cc solution was then heated with barium carbonate cream, filtered, washed, and decolorized with animal charcoal, and then titrated with decinormal alkali. This gave 11 % of free acid calculated to lactic acid. That is, 14 % of the acid was neutralized with barium carbonate.

It was thought that the amount of free lactic acid might be determined by estimating the amount of barium carbonate dissolved. To this end, about 2 grams of extract was dissolved in water and digested with barium carbonate cream on the water bath for several hours. The solution was filtered and washed, and the barium determined in the filtrate by precipitating and weighing as $BaSO_4$. The percent of barium thus found gave 3.15 % of lactic acid on

the basis of one Ba to $2C_3H_6O_3$. If it was the acid barium lactate, however, which was formed, the percent of lactic acid would be double, or 6.3%.

The determination of both free and combined lactic acid by the ether extraction method, was tried with several modifications but no satisfactory results were obtained. The results which were obtained, however, pointed unmistakably to the fact that there is a greater amount of combined than free lactic acid.

ORGANIC PHOSPHORUS, PHOSPHATES, AND COMBINED ORGANIC ACIDS.

The following described processes, it was thought, would be of value in determining the amount of organic phosphorus and the state of combination of the phosphoric acid, and in throwing some light upon the amount of combined organic acid. The process was based on the results obtained in certain qualitative tests, as follows.

First: A solution of meat extract was made slightly ammoniacal, with the production of a flocculent precipitate, which proved to be tertiary calcium phosphate, $Ca_3P_2O_8$.

Second: To the filtrate from the above precipitate was added a slight excess of an ammoniacal solution of calcium chloride, and the solution filtered from the calcium phosphate precipitated. The filtrate was divided into two portions, (a), and (b).

(a) was tested for phosphates by the addition of ammonium molybdate

with the usual precautions. filtering, treating the precipitate with ammonia, and testing the ammoniacal solution for phosphates by the addition of the "magnesia mixture ". This is in accordance with Siegfried'd statement that phosphates are precipitated from meat extract equally well by baryta water and ammoniacal calcium chloride. (8)

(b) was evaporated and ignited in a platinum dish, heated with nitric acid, and tested for phosphates with ammonium molybdate. A considerable precipitate was produced.

The following conclusions were drawn from these tests. Calcium phosphate is precipitated from a solution of meat extract by rendering alkaline with ammonia.

All phosphoric acid in the form of orthophosphates is precipitated from solution by ammoniacal calcium chloride. Organic phosphorus is present in considerable quantity.

A sample of 28.545 grams of Armour's Beef Extract was now dissolved in water and diluted to 250 cc. to be a stock solution from which aliquot portions for samples were taken. Call this solution "S". The ash was determined by the Official Method in duplicates of 25 cc. each. The alkalinity of the ash was determined by dissolving in water, filtering, and titrating the solution with decinormal sulphuric acid, using methyl orange as an indicator. The phosphates were then removed from the titrated solution by the addition of ammoniacal calcium nitrate, filtering and washing. The

filtrate was neutralized with sulphuric acid, and diluted to 250cc. Chlorine was determined on 25 cc. portions by titration with N/50 $AgNO_3$. The remaining 200 cc. of "S" was divided into two 100cc parts which were made ammoniacal, filtered and washed, and the filtrates diluted to their original volume of 200 cc. The phosphoric acid was determined in the precipitate caused by ammonia. The calcium-free stock solution, "S" was used in all following determinations. To 25 cc. portions of "S " was added from a burette an ammoniacal solution of calcium chloride in slight excess. The precipitate was allowed to settle, filtered and washed with water containing a little ammonia. The phosphoric acid was determined in the precipitate. The filtrate was heated to boiling, ammonium oxalate added in excess in order to remove the excess of calcium. Ammonium chloride and oxalate then remained in solution, but they are expelled by ignition. The precipitated calcium oxalate was filtered off and washed, the filtrate evaporated and ash determined by the Official Method. The alkalinity of the ash was determined as before. The titrated solution of ash was digested on the water bath with nitric acid for some time: the phosphoric acid was then precipitated with ammonia and calcium nitrate, filtered, washed, and the phosphoric acid determined in the precipitate. The chlorine was ~~previously~~ determined on the phosphate free ash in the manner described previously.

Now the reaction which takes place between calcium chloride and potassium phosphates is as follows:

$$2K_xH_yPO_4 + 3CaCl_2 + 2_yNH_3 = Ca_3(PO_4)_2 + 2xKCl + 2yNH_4Cl.$$

The ammonium chloride is volatilized in determining the ash, so that the additional chlorine determined is due to the potassium chloride. The following is the data and conclusions obtained from the above process.

(a) % P_2O_5 precipitated as $Ca_3P_2O_8$ by addition of NH_3	0.95
(b) % P_2O_5 " by $CaCl_2$	4.35
(a) + (b) Total % P_2O_5 as phosphates	5.30
(c) % organic phosphorus	0.30
(d) % chlorine in extract	3.60
(e) % " " " after $CaCl_2$ treatment	7.60
(f) Alkalinity of ash, as Na_2CO_3	6.90
(g) " " phosphate free ash	1.70
(e)-(d) % chlorine replacing P_2O_5	4.00

Let x = number of Cl atoms which replace I P_2O_5,

Then, xCl : P_2O_5 :: (e)-(d) ; (b)

x · 35.45 : I42 :: 4:4.35

Whence x = 3.72 Cl

$3.72K = P_2O_5$

or, $1.86K = PO_4$

or, $13K = 7PO_4$

That is, if $1K = 1PO_4(KH_2PO_4)$, then

$$12K = 6PO_4 \ (K_2HPO_4)$$

In other words, there is six times as much secondary as primary potassium phosphate.

The alkalinity of the ash is another interesting result. It is seen from the above figures that the alkalinity before removal of the phosphates is more than four times as great as it is in the phosphate free ash. This is doubtless explained by the action of the pyrophosphates formed by the ignition of the secondary orthophosphates, with the titrating acid. Thus,

$$2Na2HPO_4 = Na_4P_2O_7 + H_2O$$

$$Na_4P_2O_7 + H_2SO_4 = Na_2H_2P2O_7 + Na_2SO_4$$

The alkalinity of the phosphate free ash, however, cannot be ascribed to any form of phosphate, and is most probably due to the carbonates formed by the ignition of the salts of organic acids. The alkalinity of the phosphate free ash, as stated on the preceding page, is 1.7 % Na_2CO_3. Calculated to sodium lactate, $NaC_3H_5O_3$, we obtain 3.6 %. There is lost by ignition, therefore, nearly 2%.

In conclusion, we will state that the investigation is still in an unfinished condition. As regards the undetermined constituents, we have attempted to show that the lactic acid accounts for at least a part of it, although we have been unable to make accurate determinations of this substance. That a part, also, is a nitrogenous substance, perhaps containing phosphorus, seems quite probable.

The subject is one which requires close and persistent study, and with the rapidly increasing contributions to the chemistry of the meat extracts, we may hope soon to see the problem of the undetermined constituents satisfactorily solved.

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CHARACTERS USED IN THE TEXT.

(1), (2), (3), etc., refer to the references listed on the last page.

(a) refers to a foot-note at the bottom of the page.